



TITRIMETRIC ANALYSIS OF TANNIN FROM PROMINENT BLACK TEA BRANDS FROM INDIA

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Abstract: Tea is most popular beverage in India. It is abundant source of tannins. Tannins have ant carcinogenic and anti-mutagenic potentials which related to their ant oxidative property. Tannins are water soluble polyphenols. Black tea contains a large class of tannins known as theaflavin. Tannins are the primary flavor component, and they provide the reddish color of the tea. In the present study five most prominent back tea brands from India viz: Pariwar tea, Sapat tea, Taaza tea, Maharashtra tea, Red label tea are titrimetric analyzed for their tannin content for different time duration. Among these Pariwar black tea found highest amount of tannins while Red label black tea contain lowest amount of tannins. As most of the tea brands in India have not mention the tannin content on their packaging of all sizes, it becomes very important to determination the content of tannins because it's moderate concentration act as antioxidant but at higher concentration it may act as anti-nutritional.

Index Terms - Black Tea, Titrimetric analysis, Tannins, Tea brands

1. INTRODUCTION

Many plants possess tannins [1,2]. They are secondary phenolic metabolites from plants [3,4]. Chemically tannins are galloyl esters or oligomeric and polymeric proanthocyanidins [5]. Tannins are present as secondary polyphenolic metabolite in seed, bark, roots, stem and leaves of plants in variable range [6,7,8]. Food stuff like mango, walnuts, cashew nuts, straw berries, grapes and tea contain tannin [9]. Tea is obtained from terminal leaves of the shoots from the plant *Camellia sinensis* L. [10]. There are three main types of tea. They are black tea, oolong tea and green tea. Black tea is produced by complete fermentation of tea leaves resulting in black or brown colour of tea where catechins are oxidized by polyphenol oxidases. When any type of tea leaf is steeped in hot water it gives pungent flavor which is characteristic of tannins [11,12]. Tannins found in tea are chemically disparate, from other types of plant tannins such as tannic acid. Tannic acid is absent in tea extract [13]. In black tea, the major polyphenols are thearubigins and theaflavins [14, 15]. The major theaflavins of black tea are theaflavin, theaflavin 3-gallate, theaflavin 3'-gallate, and theaflavin 3,3'-gallate [16, 17].

Tea polyphenols are rich natural source of antioxidant. It has broad-spectrum and specific curative effects in antioxidation, anti-atherosclerosis, resistance to dental caries, antitumor, anti-radiation, anti-aging, antimicrobial and in reducing blood pressure, hematic fat, and blood sugar, and even in anti-HIV [18]. Extracts of tea leaves are also sold as dietary supplements [19]. A number of beneficial effects have been attributed to tea, including the prevention of oral cancer and tooth decay [20]. Preliminary results from an intervention study have shown that oral and topical administration of a tea preparation significantly reduced the size of oral lesions and the incidence of micronucleated oral mucosa cells in leukoplakia patients compared with a non-treated group[21]. However, these beneficial effects may be nullifying by some undesirable attributes if tannins

intake become too high. These effects include precipitation of protein, inhibition of digestive enzymes and prevention of vitamin and mineral utilization [22]. It also inhibits the absorption of both food iron and medicinal iron [23, 24]. So it becomes essential to know the amount of tannins take up by human being. The present study includes, analysis of five different prominent black tea brands from India for it's tannin content.

II. RESEARCH METHODOLOGY

2.1 Collection of sample

The 5 samples for different brands were collected from tea merchants.

Table1: Samples of tea for analysis

Sr. No.	Name of black tea brands	Number of samples	Place of collection	Packaging type
1	Pariwar	1	Nashik, Maharashtra, India	Packed
2	Sapat	1	Nashik, Maharashtra, India	Packed
3	Taaza	1	Nashik, Maharashtra, India	Packed
4	Maharashtra	1	Nashik, Maharashtra, India	Packed
5	Red label	1	Nashik, Maharashtra, India	Packed

2.2 Preparation of black tea extract

1g of tea sample was added to 25 ml of distilled water and heated for 5 minutes, 10 minutes and 15 minutes. The decoction was cooled and filtered through Whatman No.1 filter paper. The filtrate was then centrifuged at 10000 rpm for 15 minutes. The supernatant was collected in a sterile clean screw capped tube.

2.3 Qualitative estimation of tannins**1.Ferric Chloride Test:**

2-3 drops of 5% (w/v) aqueous solution of ferric chloride was added to 1 ml of extract to observe formation of greenish precipitate indicating the presence of tannins in the sample [25].

Table 2: Ferric Chloride Test

Sr. No.	Name of black tea brands	For 5min	For 10min	For 15min
1	Pariwar	+	+	+
2	Sapat	+	+	+
3	Taaza	+	+	+
4	Maharashtra	+	+	+
5	Red label	+	+	+

“+” indicates the formation of greenish precipitate and hence confirmation for presence of tannins by Ferric Chloride Test.

2. Gelatin Test:

To a 1% gelatin solution, add small amount of 10% sodium chloride. If a 1% solution of tannin is added to this gelatin solution, tannins cause white buff colored precipitation of gelatin from solution.

Table 3: Gelatin Test

Sr. No.	Name of black tea brands	For 5min	For 10min	For 15min
1	Pariwar	+	+	+
2	Sapat	+	+	+
3	Taaza	+	+	+
4	Maharashtra	+	+	+
5	Red label	+	+	+

“+” indicates the formation of buffy white precipitate and hence confirmation for presence of tannins by Gelatin Test.

2.4 Quantitative estimation of tannin:

2.4.1. Standardization of $KMnO_4$:

$KMnO_4$ is standardized by using 0.1N standard solution of oxalic acid.

2.4.2. Estimation of tannin:

Quantitative estimation of tannin was performed by titrating the extract with standard potassium permanganate solution following the method of AOAC [26] 1.25 ml aliquot of the extract was mixed with 3.1 ml of indigo-carmin solution and 93.5 ml of distilled water. This mixture was titrated against $KMnO_4$ solution ("Y" mL). As titration preceded the blue colour of the indigo-carmin passes through many shades to a final yellow with a faint pink tint at the rim. It was taken as the end-point. This volume of $KMnO_4$ was used to titrate total tannin plus all other related compounds. To determine the volume of $KMnO_4$ ("X" ml.) used to titrate non tannin (related) compound, another aliquot of 12.5 ml extract was mixed with 6.23 ml of gelatin solution, add 12.5 ml of the acidic NaCl solution and 1.2g powdered kaolin. The mixture was shaken for 15 minutes and filtered through Whatman No. 1 filter paper. 3.1ml of the filtrate was mixed with same volume of indigo carmin solution and 93.5ml of distilled water. This mixture was again titrated against $KMnO_4$ solution until colour changed to faint pink as earlier. The volume of $KMnO_4$ used to titrate true tannin was calculated by the values of Y and X. The concentration of tannin was estimated using the following relationship:

Repeat process for extract obtain after 5min, 10min and 15min boiling.

1 ml of standard $KMnO_4$ solution = 0.595 ml of 0.1N Oxalic acid

1 ml of 0.1 N Oxalic acid = 0.0042 g of tannin

III. Results and Discussion

Tannin is an important molecule that has been approved as one of the quality standard compounds for conventional tea products [27]. The different black tea brands were analyzed to reveal the percentage (%) amount of tannins at 5 minutes, 10 minutes and 15 minutes, time duration.

Table 4: Non tannin compound (X) and total tannin plus all other related compounds (Y) titration for various time duration.

Sr. no.	Name of black tea brands	5min		10min		15min	
		X	Y	X	Y	X	Y
1	Pariwar	2.3	6.3	3.9	8.7	3.3	8.4
		3.2	5.9	3.6	7.9	4.3	7.8
		2.9	6.1	4.7	8.6	3.1	7.9
2	Sapat	4.3	6.1	4.7	6.8	3.1	6.8
		3.7	5.8	3.9	6.3	2.3	6.7
		3.3	4.4	4.2	5.8	3.1	6.2
3	Taaza	1.1	4.3	2.8	5.1	3.4	6.7
		1.4	3.9	2.9	6.6	3.7	7.2
		1.4	4.7	3.2	6.4	3.5	6.9
4	Maharashtra	2.1	2.8	3.0	5.6	3.0	6.9
		2.1	3.1	3.2	6.1	2.7	6.5
		2.3	2.9	3.2	5.7	2.8	6.9
5	Red Label	2.9	4.8	3.2	5.5	3.1	5.5
		2.8	4.9	3.3	5.3	3.3	5.4
		3.1	4.8	3.2	5.3	2.9	5.6

A= volume of $KMnO_4$ used to titrate true tannin

X= Non tannin related compound

Y= Total tannin of all others compound

Volume of KMnO_4 used to titrate true tannin (A) = Y-X

Table 5: Volume of KMnO_4 used to titrate true tannin (A)

Sr.No.	Name of black tea brands	5min (A)	10min (A)	15min (A)
1	Pariwar	4.4	4.8	5.1
		2.7	4.3	3.5
		3.2	3.9	4.8
2	Sapat	1.8	2.1	3.7
		2.1	2.4	4.4
		1.1	1.6	3.1
3	Taaza	3.2	2.3	3.3
		2.5	3.7	3.5
		3.3	3.2	3.7
4	Maharashtra	0.7	2.6	3.8
		1.0	2.9	3.8
		0.6	2.5	4.1
5	Red label	1.9	2.3	2.4
		2.1	2.0	2.1
		1.7	2.3	2.7

Volume of equivalent amount of Oxalic acid (B)

Volume of equivalent amount of Oxalic acid (B) = $A \times 0.595/1$

grams of tannins (C)

gm of tannins (C) = $B \times 0.0042/1$

Percentage of tannins (%) (D)

Percentage of tannins (%) (D) = $100 \times C/1$

Table 6: Analysis of 5 minutes boiling extract aliquots.

Sr. No.	Name of black tea brands	Mean (A) ml Volume of KMnO_4 used to titrate true tannin	(B) ml Volume of equivalent amount of Oxalic acid	(C) gm Gm of tannins	(D) % % of tannins
1	Pariwar	3.4	2.023	0.0084966	0.84
2	Sapat	1.6	0.952	0.0039984	0.39
3	Taaza	3.0	1.785	0.007497	0.74
4	Maharashtra	0.7	0.4196	0.001749	0.17
5	Red Label	1.9	1.1305	0.00474	0.47

Table 7: Analysis of 10 minutes boiling extract aliquots.

Sr. No.	Name of black tea brands	Mean (A) ml Volume of KMnO ₄ used to titrate true tannin	(B) ml Volume of equivalent amount of Oxalic acid	(C) gm Gm of tannins	(D) % % of tannins
1	Pariwar	4.3	2.5585	0.01074	1.07
2	Sapat	2.0	1.19	0.004998	0.49
3	Taaza	3.0	1.785	0.007497	0.74
4	Maharashtra	2.6	1.547	0.0064974	0.64
5	Red Label	2.2	1.309	0.0054978	0.54

Table 8: Analysis of 15 minutes boiling extract aliquots.

Sr. No.	Name of black tea brands	Mean (A) ml Volume of KMnO ₄ used to titrate true tannin	(B) ml Volume of equivalent amount of Oxalic acid	(C) gm Gm of tannins	(D) % % of tannins
1	Pariwar	4.4	2.618	0.01099	1.09
2	Sapat	3.7	2.2015	0.009246	0.92
3	Taaza	3.5	2.0825	0.0087465	0.87
4	Maharashtra	3.9	2.3205	0.009746	0.97
5	Red Label	2.4	1.428	0.005997	0.59

Table 9: Absolute analysis of black tea brands to reveal % of tannins for different time duration.

Sr. no.	Name of black tea brands	5 minutes (D) = % of tannins	10 minutes (D) = % of tannins	15 minutes (D) = % of tannins
1	Pariwar	0.84	1.07	1.09
2	Sapat	0.39	0.49	0.92
3	Taaza	0.74	0.74	0.87
4	Maharashtra	0.17	0.64	0.97
5	Red Label	0.47	0.54	0.59

It was found that irrespective of brands, percentage of tannins increased with time duration of boiling sample black tea brand. Pariwar black tea brand had highest amount of percentage of tannins and Red Label tea had lowest amount of percentage of tannins. Sapat, Taaza, and Maharashtra black tea brands showed moderate amount of tannins. and Red Label tea had lowest amount of percentage of tannins. Sapat, Taaza, and Maharashtra black tea brands showed moderate amount of tannins

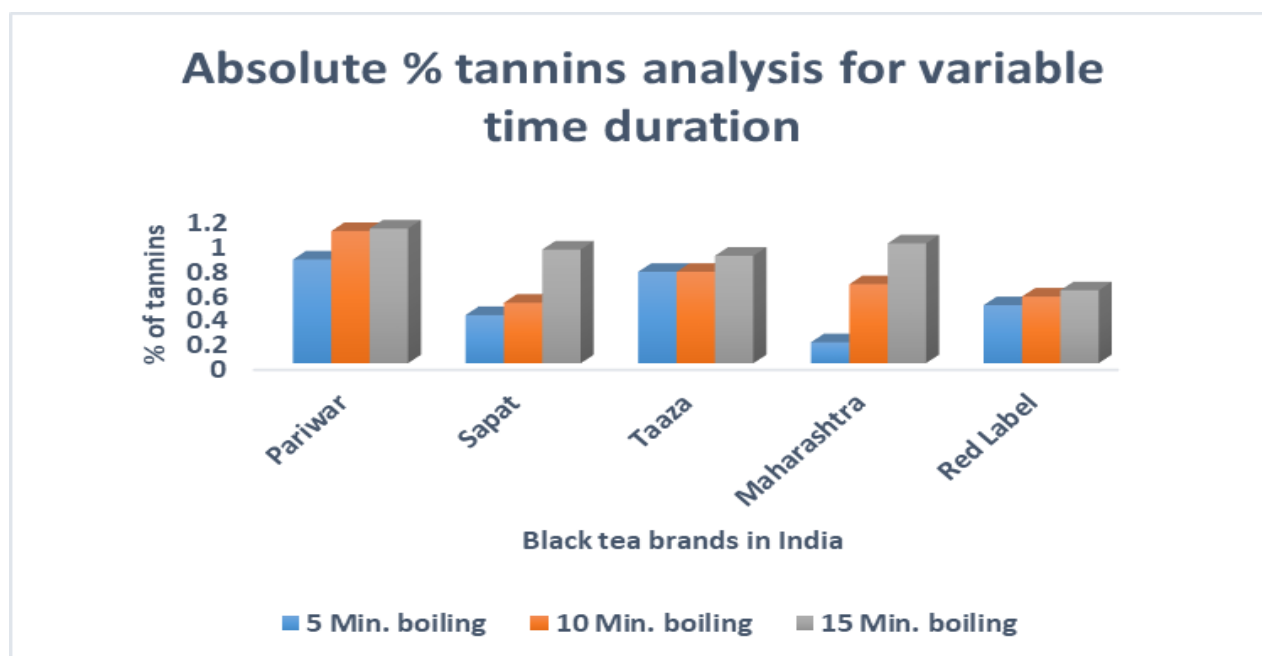


Figure 1: Absolute % tannins analysis for variable time duration

IV. Conclusion

The present study shows that from 5 black tea brands samples under analysis, 3 named Sapat, Taaza and Maharashtra tea have moderate percentage of tannins and Pariwar and Red label black tea brand samples have highest and lowest percentage of tannins respectively. The percentages of tannins are very important information as it is beneficial only when consumed in limited amount and the higher consumption may give adverse effect on health of human being.

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